# HSB Project 5

# Aging and Spontaneous Disease Phenotypes in Selected Inbred Strains

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***Background and Rationale***
Susceptibility to many complex disease traits, including cancer and cardiovascular disease is heritable (Stankiewicz and Lupski, 2006). These diseases have complex etiologies that are considered quantitative or continuous because the number of allelic variants or mutations in many different genes carried in the germline that are associated with the pathogenesis of the disease. Environmental stressors, including chemical pollutants, somatic mutations, and nutrition may also significantly influence the prevalence and severity of a disease. Research has shown that inherited single nucleotide polymorphism and gene copy number variations (genetics) as well as inherited and/or altered methylation patterns in critical genes (epigenetics) in individuals are believed to have significant roles in disease susceptibility and prevalence***.*** Animal models for human disease must also incorporate genetic and epigenetic diversity in order to allow investigation of disease phenotypes and association with genic and nongenic sequences that are common to both species (Harrill *et al.*, 2009). From the NTP/NIEHS-Perlegen resequencing project we have learned a great deal in regard to the strengths and weaknesses of laboratory inbred mouse strains compared to inbred wild derived lines (Frazer *et al.*, 2007; Yang *et al.*, 2007).

We selected 9 inbred strains based upon their known genetic diversity and the NTP B6C3F1/J reference strain to phenotype and characterize spontaneous disease traits in order to prepare for multiple inbred strains studies of NTP nominated chemicals for toxicology and carcinogenesis. We estimate that we can significantly increase our power of detection of environmental exposure related disease that may vary significantly based upon strain differences in absorption, disposition, metabolism, and excretion of parent and metabolite(s) that affect bioavailability and target organ toxicity, pathogenesis, and disease susceptibility.

***Key Issues***

- Selection of appropriate mouse strains as models for human genetic diversity and disease susceptibility in response to environmental toxicant exposures.

- Study design and conduct used for detecting variable ranges of response to sporadic or spontaneous disease and xenobiotic exposure to multiple strains of inbred mice to model potential xenobiotic induced human disease in a gene x environment interaction paradigm of human relevance.

Eight of the strains selected are from the eight-way collaborative cross used to produce recombinant inbred (RI) lines that are a random mixture of the 8 parental lines

representative of the three mouse subspecies without any introgression into the wild-derived lines (Churchill *et al.*, 2004; Roberts *et al.*, 2007). We included the wild-derived inbred strain (PWK/EiJ) along with the B6C3F1 for reference for the NTP historical database to complete this cohort. These RI lines are designed to aid quantitative trait analysis by their fine mosaic structure that will aid positional mapping and identification of candidate genes associated with the trait of interest. Power calculations have indicated that a significant increase in detection of potential carcinogens when multiple strains are used (5 or more) when the test agent is not highly penetrant across all strains. Penetrance may be due to toxic potency of the test agent and strain specific susceptibility or resistance alleles present in the selected species and strain.

Statistical calculations have been completed that allow estimation of power to detect chemical related outcomes across multiple rodent strains based upon expected discordance in outcomes with less potent test agents. Differences between species and strains in response to an exposure may be due to chemical potency to induce toxicity (Tennant, 1993). Based upon extensive NTP experience with the 4-cell assay (2 species and 2 sexes per species) there is concern over the potential for type 1 and type 2 errors and human relevance. Studies with carcinogens with low penetrance often promote tumorigenesis in target tissues consistent with high background rates of strain and tissue specific susceptibility that are of unknown human relevance. By increasing the number of strains and target tissue spectrum of susceptibility, we can increase the power of detection and work toward reduction of type 1 and 2 errors

***Hypothesis***

The genetic diversity in the 10 strains selected will show significant age-related range of spontaneous disease and functional phenotypes that will aid selection of strains for toxicology and carcinogenesis research and testing.

***Approach and Specific Aims***

Aging and sporadic disease phenotyping in 10 genetically-diverse stains of mice (including the B6C3F1 mouse) are being used to establish an NTP reference data base for survival, disease phenotypes, and temporal changes over time in heart, respiration, and genetic and/or epigenetic measurements to aid design of 2-year toxicology and carcinogenicity studies in multiple genetically diverse strains to test chemicals nominated to the NTP. Two additional cohorts will be tested independently in support creation of this benchmark reference database. The special studies are designed to: (1) develop biomarkers of aging and disease in specific tissues and organs at 26, 52, 78, and 104 wks of age using clinical pathology (hematology and chemistry) and genetical genomics (Jansen and Nap, 2001) and (2) determine functional phenotypes of the cardiovascular and respiratory systems at 26, 52, and 78 weeks of age.

The strains to be evaluated for aging and spontaneous disease are: 129/SvlmJ, A/J, C3H/HeJ, C57BL/6J, CAST/EiJ, NOD.B10H2$^{b}$/LtJ, NZO/HiLtJ, PWK/PhJ, WSB/EIJ, and B6C3F1/J. The congenic NOD.B10H2$^{b}$/LtJ was selected over the NOD/LtJ line in order to improved survival that approximates the other lines but provides significant genetic diversity.

### *Significance and Expected Outcomes*

An NTP public benchmark reference database can aid scientists in selecting the most suitable strains for chemical toxicology and disease studies for extrapolation across species. Further, the magnitude of the observed differences in toxicity and disease responses across strains may provide quantitative data for risk assessment uncertainty factors that might also replace some current default assumptions with quantitative estimates based upon observed genetic diversity in the mouse models for the diverse human population.

### *Current Activities*

Aging and survival analysis (spontaneous disease) studies are in progress. Protocols for identifying and developing biomarkers at specific life stages, and functional analysis of cardiopulmonary functions are in progress and under peer-review.

### *Future Plans*

Study designs for toxicology and carcinogenesis studies in multiple strains are in progress. Toxicology and carcinogenesis studies will be performed with the total number of mice used per exposure group fractioned based upon the number of strains used to increase genetic diversity. For example, a study using 80 mice/sex/strain/exposure can be fractionated to 10 mice sex/strain/exposure group for the 8 strains selected to achieve maximum statistical power for detection of a potential carcinogen. A positive outcome observed in one or more of the cell(s) of a 16-cell design would increase our confidence in regard to a potential false positive if only a single strain is used. All negative in a 16-cell study (8 strains x 2 sexes x 1 species) with significant power of detection will increase our confidence for a potential false negative (type 2 error) outcome if only a single strain is used. More statistical research for optimization of study design is required that account for other predictors, e.g. mode/mechanisms of action, toxic potency, exposure route, and rate of exposure, etc.

### *References*

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